EXHIBIT E

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Collins et al.	Art Unit:	1807
Serial No:	08/283,080	Examiner:	Rees, D.
Filed:	May 3, 1994		
For:	TARGET AND BACKGROUND CAPTURE METHODS WITH AMPLIFICATION FOR AFFINITY ASSAYS	Docket No:	2583511

PRELIMINARY AMENDMENT AND RESPONSE TO RESTRICTION REQUIREMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Please amend the application as follows.

In the Specification:

Please insert the following as the first sentence of the specification following the title:

This is a ~~divisional~~ continuation of U.S. Serial No. 08/400,657 filed March 8, 1995.

Please cancel Claims ~~1-24~~ and replace them with the following new Claims 25-50:

25. A method for amplifying a target polynucleotide contained in a sample comprising the steps of:

(a) contacting the sample with a first support which binds to the target polynucleotide;

(b) substantially separating the support and bound target polynucleotide from the sample; and

(c) amplifying the target polynucleotide.

2 26. The method of Claim 25 wherein the first support is retrievable.

3 27. The method of Claim 25 wherein the first support includes a probe which binds with the target polynucleotide.

4 28. The method of Claim 25 wherein the target polynucleotide is amplified with a polymerase.

5 29. The method of Claim 28 wherein the polymerase is a DNA polymerase, an RNA polymerase, a transcriptase or Qβ replicase.

6 30. The method of Claim 28 wherein the target polynucleotide is a DNA polynucleotide and the polymerase is a DNA polymerase.

007 7 31. A method for detecting a target polynucleotide contained in a sample comprising the steps of:

(a) contacting the sample with a first support which binds to the target polynucleotide;

(b) substantially separating the first support and bound target polynucleotide from the sample;

(c) amplifying the target polynucleotide; and

(d) detecting the presence of the amplified target polynucleotide.

8 32. The method of Claim 31 wherein the first support is retrievable.

9 33. The method of Claim 31 wherein the first support includes a probe which binds with the target polynucleotide.

10 34. The method of Claim 31 wherein the target polynucleotide is amplified with a polymerase.

11 ~~35~~. The method of Claim ~~34~~ 10 wherein the polymerase is a DNA polymerase, an RNA polymerase, a transcriptase or Qβ replicase.

12 ~~36~~. The method of Claim ~~35~~ 11 wherein the target polynucleotide is a DNA polynucleotide and the polymerase is a DNA polymerase.

13 ~~37~~. The method of Claim ~~31~~ 7 wherein the amplified target polynucleotide is contacted with a label.

14 ~~38~~. The method of Claim ~~31~~ 7 wherein the amplified target polynucleotide is contacted with a labeled probe.

15 ~~39~~. The method of Claim ~~31~~ 7 wherein the amplified target polynucleotide is contacted with a second support which binds to the amplified target polynucleotide.

16 ~~40~~. The method of Claim ~~39~~ 15 wherein the amplified target polynucleotide is contacted with a labeled probe.

17 ~~41~~. The method of Claim ~~40~~ 14 wherein the target polynucleotide is amplified with a polymerase.

18 ~~42~~. The method of Claim ~~41~~ 17 wherein the target polynucleotide is a DNA polynucleotide and the polymerase is a DNA polymerase.

~~43~~. A method for detecting a target polynucleotide contained in a sample comprising the steps of:

(a) contacting the sample with a first support which binds to the target polynucleotide;

(b) substantially separating the first support and bound target polynucleotide from the sample;

(c) amplifying the sample with a DNA polymerase;

(d) contacting the amplified target polynucleotide with a second support which binds to the amplified target polynucleotide and a labeled probe which binds to the target polynucleotide; and

(e) detecting the presence of the amplified target polynucleotide.

44. A kit for detecting a target polynucleotide contained in a sample comprising:

(a) means for substantially separating the target polynucleotide from the sample;

(b) means for amplifying the target polynucleotide;

(c) means for binding the amplified target polynucleotide to a solid medium; and

(d) means for labeling the amplified target polynucleotide.

45. The kit of Claim 44 wherein:

(a) the means for substantially separating the target polynucleotide from the sample include a first support;

(b) the means for amplifying the target polynucleotide include a polymerase;

(c) the means for binding the amplified target polynucleotide to a solid medium include a second support which binds to the amplified target polynucleotide; and

(d) a detector probe for labeling the amplified target polynucleotide.

22 ~~46~~ 21. The kit of Claim ~~45~~ further comprising a capture probe which binds to the first support and to the target.

23 ~~47~~ 22. The kit of Claim ~~46~~ wherein the polymerase is a DNA polymerase and the detector probe is labeled.

24 ~~48~~. A kit for amplifying a target polynucleotide contained in a sample comprising:

(a) means for substantially separating the target polynucleotide from the sample and

(b) means for amplifying the target polynucleotide.

25 ~~49~~. The kit of Claim ~~48~~ 24 wherein:

(a) the means for substantially separating the target polynucleotide from the sample includes a support which binds to the target polynucleotide and

(b) the means for amplifying the target polynucleotide includes a polymerase.

~~50~~ 26. The kit of Claim ~~49~~ 25 wherein:

(a) the polymerase is a DNA polymerase; and

(b) the means for substantially separating the target polynucleotide from the sample includes a probe which binds to the target polynucleotide and the support.

REMARKS

I. Status Of The Application

A. The Application Is A Divisional Of U.S. Serial No. 08/400,657.

The subject application (Serial No. 08/283,080) was filed as an original application. However, as discussed herein, the application is entitled to the benefit of consideration as a divisional application to copending application U.S. Serial No. 08/400,657 filed March 8, 1995. U.S. Serial No. 08/400,657 is itself a continuation application to U.S. Serial No. 08/257,469, filed June 8, 1994 and now abandoned. U.S. Serial No. 08/257,469 is a continuation application to U.S. Serial No. 08/124,826 filed September 21, 1993 and now abandoned. Thus, U.S. Serial No. 08/400,657 claims priority from U.S. Serial No. 08/124,826 filed September 21, 1993.

Applicants are permitted to cross-reference the subject application with and claim the benefit of the earlier priority date of Serial No. 08/400,657 and U.S. Serial No. 08/124,826 pursuant to 37 CFR §1.78. Specifically, 37 CFR §1.78(a)(1) provides that a nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications. In order for this to be proper, each prior filed copending application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 USC §112. In addition, each prior application must be:

(i) Complete as set forth in §1.51(a)(1), or

(ii) Entitled to a filing date as set forth in §.53(b)(a), §1.60 or §1.62 and include the basic filing fee set forth in §1.16; or

(iii) Entitled to a filing date as set forth in §1.53(b)(1) and have paid therein the processing and retention fee set forth in §1.21(1) within the time period set forth in §1.53(d)(1).

Finally, as required by 37 CFR §.78(a)(2), a nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number. As discussed herein, the subject application satisfies these conditions.

First, neither application claims status as a provisional application so that §1.78(a)(1) and (2) are the applicable provisions of §1.78. Next, both the subject application and Serial No. 08/400,656 name Mark Collins as an inventor. Next, the priority application (Serial No. 08,400,656) discloses Collins' invention as claimed in at least one claim of the later filed application (the subject application). This is true because Serial No. 08/400,656 and Serial No. 08/124,826 are continuations-in-part applications to U.S. Serial No. 07/136,920, which is substantively identical to the subject application and specifically incorporated by reference in Serial No. 08/400,656 (See the first sentence of the priority application of Serial No. 08/400,656 enclosed herewith as Appendix 1). Next, Serial No. 08/400,656 and Serial No. 08/124,836 were complete as set forth in §1.51(a)(1). Finally, Applicants have amended the specification to contain in the first sentence following the title a reference to Serial No. 08/477,656, identifying it by application number.

B. U.S. Serial No. 08/400,657 claims priority from U.S. Serial No. 07/136,920.

As discussed, Serial No. 08/400,657 is itself a continuation application of U.S. Serial No. 08/257,469 filed June 8, 1994. Moreover, Serial No. 08/257,469 is a continuation application of U.S. Serial No. 08/124,826 filed September 21, 1993 and now

abandoned. Serial No. 08/124,826 is a continuation application of U.S. Serial No. 946,749 filed September 17, 1992 and now abandoned. Serial No. 946,749 is a continuation application of U.S. Serial No. 07/648,468 filed January 31, 1991 and now abandoned. Serial No. 07/648,468 is a continuation-in-part application of U.S. Serial No. **07/136,920** filed December 21, 1987 and now abandoned. Serial No. **07/136,920** is a continuation-in-part application of U.S. Serial No. 06/922,155 filed October 23, 1986 and now abandoned. The disclosures of both of Serial No. **07/136,920** and 06/922,155 are incorporated into the subject application by reference.

C. The Application Is Substantively Identical To U.S. Serial No. 07/944,505[1].

The subject application (Serial No. 08/283,080) is also substantively identical to U.S. Serial No. 07/944,505, filed September 14, 1992 and now abandoned. Serial No. 07/944,505 is a continuation application to U.S. Serial No. 07/644,967 filed January 22, 1991 and now abandoned. Serial No. 07/644,967 is a continuation application to U.S. Serial No. **07/136,920** filed December 21, 1987 and now abandoned. As mentioned, Serial No. **07/136,920** is a continuation-in-part application to U.S. Serial No. 06/922,155 filed October 23, 1986 and now abandoned. U.S. Serial No. **07/136,920** and No. 06/922,155 are the same applications which begin the chain of applications described earlier as underlying U.S. Serial No. 08/400,657, and incorporated by reference therein.

D. The Application Is Subject To A Restriction Requirement.

The subject application has not yet been examined but is subject to a restriction requirement mailed September 5, 1995. The subject application comprises 24 claims. Claims 1-20 and 22-23 are nominally to methods for amplifying target polynucleotides. Claim 21 is to a kit for capturing and amplifying a target polynucleotide. Claim 24 is to an instrument for performing assays for target nucleotides. The Examiner has found two groups of inventions. Group I comprises Claims 1-23 drawn to a method of amplification

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[1]As a point of clarification, Applicants had intended to file the subject application as a continuation application to U.S. Serial No. 07/944,505. However, Applicants inadvertently lost copendency between the two applications thereby necessitating this alternative action.

of a target polynucleotide. Group II comprises Claim 24 is drawn to an instrument for assaying for target polynucleotides. In light of the newly amended claims, Applicant elects the claims to methods and has cancelled Claim 24 from the application. Similarly, U.S. Serial No. 07/944,505 was examined and subjected to a restriction requirement. Claims 1-23 were elected for prosecution. Claim 24 was withdrawn from consideration. Claims 1-23 were examined and rejected in an Office Action mailed November 5, 1992. As will be discussed more fully herein, this Preliminary Amendment is submitted to address the Office Action mailed November 5, 1992.

II. The Official Action Mailed November 5, 1992 For U.S. Serial No. 07/944,505

A. The status of U.S. Serial No. 07/944,505

U.S. Serial No. 07/944,505 included the same 24 claims filed with the subject application. The application was examined and an Official Action was mailed November 5, 1992. A copy of the action is enclosed for the Examiner's benefit as Appendix 2. Claims 1-24 were subject to a restriction requirement. Claim 24 was withdrawn from consideration. Claims 1-23 were elected and examined. All of Claims 1-23 were rejected and the rejection was made final. Applicants' Preliminary Amendments and Remarks herein are intended to respond to the Official Action mailed November 5, 1992.

B. The Rejections Under §112

Claims 1-23 of Serial No. 07/944,505 were rejected under 35 USC §112, second paragraph as being indefinite. Claim 1 and others were rejected for reciting the phrase "support capable of specifically associating with the target under binding conditions." The Examiner determined the phrase is vague and indefinite functional language describing a chemical moiety by what it does rather than by what it is structurally so that it is impossible to know what is and what is not claimed. Claim 6 was rejected for its use of the term "probe". The Examiner determined this usage is vague and indefinite. The Examiner asked whether Applicants intend a specific nucleic acid sequence which will probe through hybridization or whether something else is intended. Claim 6 was also rejected for being phrased in functional language.

Claim 10 was rejected for reciting the term transcriptase. This use was said to be vague and ambiguous. The Examiner asked whether reverse transcriptase was intended? Claim 11 and others were rejected for reciting the phrase "non-specific oligonucleotide primer." Claim 13 and others were rejected for their use of the phrase "substantially separating." These phrases were said to be vague and indefinite.

Claim 21 was rejected for its use of the phrase "capable of binding to a retrievable support." The claims were rejected for reciting the phrase "retrievable support." These phrases were also said to be vague, indefinite and functional language. In addition, the Examiner determined it is not clear what support would not be retrievable so that this usage is confusing. The claims were also rejected for their usage of the phrase "reagents adapted to be applied to said removal product." This phrase was said to be vague and indefinite.

Claims 22 and 23 were rejected for reciting the phrase "the method of Claim 21." The Examiner noted that Claim 21 is to a kit rendering Claims 22 and 23 confusing. Claim 23 was also rejected for its use of the phrase "capable of interacting with a magnetic field." This phrase was said to be vague and indefinite in light of the known ability of any carbon, nitrogen or hydrogen containing compound to interact with a magnetic field (citing NMR). The Examiner determined it is not clear what Applicants are describing.

C. The Rejections Under §103

Claims 1-23 were rejected under 35 USC §103 as unpatentable over Mullis U.S. No. 4,683,202 when taken with any one of Moss, Stabinsky or Engelhardt and taken further in view of Ranki, Josephson or Schroder if necessary.

Mullis was said to teach DNA amplification and point out the great value of this method for improved sensitivity and improved ability to isolate specific nucleotide sequences. It was admitted, however, that Mullis does not specifically teach nucleic acid affinity chromatography prior to the amplification reaction. At the same time, all of the secondary references were said to teach the well known method of affinity chromatography, both with nucleic acid attached to a support (direct hybridization) as well as through ligands attached to one strand of nucleic acid (e.g., biotin-avidin). The secondary references were further said to teach the value of affinity chromatography in its ability to isolate specific nucleotide sequences and remove unwanted sequences which

would interfere with later usefulness of the sequences. The secondary references also teach the greater efficiency of hybridization and improved sensitivity of an affinity purified sample compared to a non-purified sample (citing Moss, Fig. 3), although the Examiner asserted this fact would be well known to one of ordinary skill in the art. It would be obvious, the Examiner concluded, for one of ordinary skill to combine the teachings of the primary reference (Mullis) which show improved sensitivity and improved ability to purify a sequence with the secondary references which teach a method providing improved ability to purify a sequence and improved sensitivity since the methods are all directed to the same result and one of ordinary skill would expect an improvement in results.

As to claims directed to association with a "probe," the Examiner asserted it is not clear what is meant by this language (apparently referring to the prior discussion under §112). However, the Examiner continued, it appears to be the well known method of sandwich hybridization (citing Ranki) which also claims increased sensitivity and greater ability to isolate specific sequences.

As to claims reciting "non-specific oligonucleotide primer," the Examiner asserted it is not clear what is meant by this language. At the same time, however, the Examiner asserted that it appears that Applicants are simply referring to the well known method of random primer polymerization which is used to label probes. The Examiner asserted this method is well known not only as an efficient method of making a second copy (into which label nucleotides can be added) but is also more efficient than using a single primer. The Examiner concluded one of ordinary skill in the art would have known this technique and would have been motivated to use it since it makes a second strand thereby doubling the number of copies to be amplified.

As to claims using a "bead capable of interacting with a magnetic field," the Examiner asserted it is not clear what it meant by this language. At the same time, however, the Examiner asserted it appears to be the well known method of Josephson and Schroder for magnetic separations.

As to the kit claims, the Examiner concluded it would have been obvious to one of ordinary skill in the art to package all of the components in a kit for the convenience of practitioners of the method.

For clarification, the Examiner stated it as his position that Applicants simply

combined the well known method of nucleic acid amplification with the equally well known method of affinity chromatography to produce a result which would have been expected and with sufficient motivation to make the combination. The Examiner concluded Applicants' invention would have been prima facie obvious to one of ordinary skill in the art at the time of the invention.

Finally, the Examiner noted that Wood, Noyes and Shih are merely cumulative to the teachings of Moss, Stabinsky, Engelhardt and that the two other Mullis references were merely cumulative to Mullis.

D. The Restriction Requirement

The Examiner reminded Applicants that the application was subject to a restriction requirement whereby Applicants were directed to elect from between the claims of Group I (Claims 1-23) and Group II (Claim 24). The Examiner further reminded Applicants that they had provisionally elected to prosecute the claims of Group I. Finally, the Examiner instructed Applicants that they must affirm their provisional election of Group I in responding to the Office Action mailed November 5, 1992.

III. Applicants' Invention

Before addressing Applicants' amendments and the merits of the Official Action mailed November 5, 1992 for Serial No. 07/944,505, Applicants believe it would be useful to describe the invention disclosed and claimed in the subject application.

Applicants' invention pertains to improved methods and kits for use in capturing, amplifying and detecting target molecules. Embodiments of the invention provide methods for rapid, sensitive detection of nucleic acid targets present in clinical samples. The invention permits capture, amplification and detection of DNA and RNA targets present in clinical samples in extremely small amounts and with great sensitivity. The invention couples target amplification techniques with noise reduction techniques to provide a detection method of great sensitivity. At the same time, the invention permits the production of large amounts of purified target polynucleotides. Diagnostic embodiments of the invention are readily adaptable to automation and a variety of labeling techniques.

Nucleic acid hybridization is well known for its utility in assays for the detection of various pathogens and diagnosis of various diseases. The invention extends this utility into regimes of assay sensitivity previously unavailable to the practitioner. The invention advances nucleic acid hybridization methods by combining target purification methods with target amplification methods. Nucleic acid targets can be amplified using any of a variety of methods. These include the polymerase chain reaction PCR. However, although PCR was introduced with the promise of great specificity for target amplification, the reality is that PCR is not as specific as practitioners require. Due to nonspecific binding of PCR primers, PCR will often amplify nucleic acids other than the nucleic acid of interest.

Applicants have found that target purification methods can be applied prior to target amplification to insure that only the intended target polynucleotides are amplified. More fundamentally, minute amounts of target polynucleotides can be amplified exponentially in substantially purified form.

IV. Applicants' Response To The Official Action Mailed November 5, 1992 For Serial No. 07/944,505

A. Applicants' Amendments

Applicants would request entry of the amendments submitted herewith. Applicants have cancelled Claims 1-24 and replaced them with new Claims 25-50. The new claims are submitted to overcome the rejections made in the November 5, 1992 Office Action for Serial No. 07/944,505. At the same time, the new claims feature the various embodiments of the invention described supra. Applicants' amendments add no new matter to the application.

B. Response To Restriction Requirement

U.S. Serial No. 07/944,505 was subject to a restriction requirement. The invention was restricted into two have groups. Group I contained Claims 1-23; Group II contained Claim 24. Applicants had previously provisionally elected to prosecute the claims of Group I. Thereafter, Applicants were directed to affirm their election in responding to the Office Action mailed November 5, 1992. Applicants would hereby affirm their provisional election to prosecute the claims of Group I.

C. Response To Rejections Under §112

As described, previously, Claims 1-23 were rejected Under 35 USC §112, second paragraph as indefinite. Applicants traverse all of these rejections as inapplicable to the newly added claims and overcome as to Claims 1-23 because these claims have been canceled. More specifically, Claim 1 and others were rejected for reciting the phrase "support capable of specifically associating with the target under binding conditions." The Examiner determined the phrase is vague and indefinite. Although Applicants do not necessarily agree with the Examiner's determination, the rejection is now unfounded because the phrase is not included in the claims now under consideration.

Claim 6 was rejected for its use of the term "probe." The Examiner asked whether Applicants intend a specific nucleic acid sequence which will probe through hybridization or whether something else is intended. Claim 6 was also rejected as phrased in functional language, presumably for its recitation of the phrase "support is capable of associating with the target through a probe." Applicants submit this phrase is nowhere used in the claims now under consideration. Therefore, this part of the rejection of Claim 6 is now unfounded. Applicants submit the rejection as to Applicants' use of the term "probe" is clearly misplaced. Applicants have specifically defined the term "probe" at page 1, line 36 - page 2, line 3 of the specification. Additionally, Applicants have disclosed numerous examples of probes throughout the application (See, e.g., page 23, line 17 et seq and figure 2; page 28, line 10 et seq and figures 4-6; page 35, line 16 et seq. and the examples). In light of these, Applicants submit their use of the term "probe" is not vague and indefinite and request that this rejection be withdrawn.

Claim 10 was rejected for reciting the term "transcriptase." The Examiner asked whether "reverse transcriptase" was intended. Applicants note that the term transcriptase generally applies to a DNA dependent RNA polymerase and the term reverse transcriptase generally applies to an RNA dependent DNA polymerase. Therefore, the terms "transcriptase" and "reverse transcriptase" generally apply to different polymerases. (See, e.g., Dictionary of Biochemistry of Stenesh, Wiley - Interscience (1975).) Applicants also note that the use of transcriptase is disclosed in the specification at page 2, line 35 - page 3, line 2. Similarly, the specification discloses the use of reverse transcriptase at page 29, lines 6-10. Accordingly, Applicants' amended claims recite the use of both transcriptase

and reverse transcriptase. Applicants further submit their use of the term transcriptase is not vague and indefinite. Applicants respectfully request that this rejection be withdrawn.

Claim 11 and others were rejected for reciting the phrase "non-specific oligonucleotide primer." While Applicants do not necessarily agree with the rejection, Applicants submit the rejection is inapplicable to the amended claims because these claims do not recite the phrase. Accordingly, Applicants request that the rejection be withdrawn.

Claim 13 and others were rejected for their use of the phrase "substantially separating." Applicants submit the rejection is misplaced. Applicants have disclosed the use of retrievable supports in the methods and kits of their invention. These retrievable supports can be particles, grains, beads or filaments capable of dispersion within and separation from a medium (See page 11, lines 26-29). Magnetic beads are preferred as retrievable supports (See; page 9, line 16 et seq.). Applicants submit that it is reasonable and accurate to describe the separation of such supports as "substantially separating." Applicants submit that those with skill in the art will have no difficulty in understanding Applicants' use of the phrase in the claims. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claim 21 was rejected for its use of the phrase "capable of binding to a retrievable support" and "retrievable support." Applicants submit the rejections are misplaced. Applicants have specifically defined the terms "retrievable" (page 2, lines 13-16) and "support" (page 2, lines 17-19) in the specification. Additionally, Applicants have identified numerous examples of retrievable supports in the specification such as magnetic beads (page 9, line 17 et seq.) particles, grains or filaments capable of dispersion and separation from a medium (page 11, lines 26-32). Applicants submit that those skilled in the art will have no difficulty in understanding what is meant by "retrievable support" in light of these express definitions and numerous examples. Accordingly, Applicants request that this rejection be withdrawn. Additionally, while Applicants do not necessarily agree with this rejection, Applicants note that the amended claims do not recite the phrase "capable of binding to a retrievable support." Therefore, Applicants submit the rejection is inapplicable to the amended claims and request that it be withdrawn.

Claim 21 was also rejected for its use of the phrase "reagents adapted to be applied to said removal product." Again, while Applicants do not necessarily agree with this

rejection, Applicants note that the phrase is not used in the amended claims. Accordingly, Applicants submit the rejection is inapplicable to the amended claims.

Claims 22 and 23 were rejected for reciting the phrase "method of Claim 21." The Examiner noted that Claim 21 is to a kit. Applicants submit that this rejection is inapplicable to the amended claims and request that it be withdrawn.

D. Response To Rejections Under §103

As described supra, Claims 1-23 were rejected under 35 USC §103 as obvious. In concluding the rejection, the Examiner took the position that Applicants have simply combined the well known method of nucleic acid amplification with the equally well known method of affinity chromatography to produce an expected result. The Examiner further asserted the art provided sufficient motivation for the combination. The Examiner concluded the invention would have been prima facie obvious to one of ordinary skill at the time it was made. Applicants traverse these rejections. Applicants submit no proper prima facie argument for obviousness can be made out and that in purporting to do so the Examiner misapplied the teachings of the cited references, ignored other teachings within the primary references which teach away from Applicants' invention and did not consider the teachings of other references, not of record, which evidence that the invention addresses a problem present in the art but not solved by others prior to Applicants' invention.

Claims 1-23 were rejected as unpatentable over Mullis (U.S. No. 4,683,202) when taken with any one of Moss, Stabinsky or Engelhardt and taken further in view of Ranki or Josephson or Schroder if necessary. Wood, Noyes and Shih were said to be cumulative to Moss, Stabinsky and Engelhardt. Mullis (U.S. No. 4,683,195) and Mullis (Cold Spring Harbor Symbosia) were said to be cumulative to Mullis (U.S. No. 4,683,202).

Mullis was said to teach DNA amplification and point out the great value of this method for improved sensitivity and improved ability to isolate specific nucleotide sequences. The Examiner admitted Mullis does not specifically teach nucleic acid affinity chromatography prior to amplification. The secondary references were cited for teaching various aspects of affinity chromatography in general and the value of affinity chromatography in isolating specific sequences from unwanted sequences. Moss was

particularly cited for teaching that greater hybridization efficiency and improved sensitivity could be obtained for an affinity purified sample in comparison to an impure sample. The Examiner concluded it would be obvious to combine the teachings of Mullis (improved sensitivity and improved purification) with the secondary references (improved purification and improved sensitivity) since the methods are all directed to the same result and one of ordinary skill would expect an improved result.

Applicants submit the Examiner's conclusion is the product of an improper picking and choosing of selective disclosure from the cited references to obtain Applicants' invention and that when the references are considered for all that they teach the references do not disclose or suggest Applicants' invention. For example, while it is true that Mullis (U.S. No. 4,683,202) discloses DNA amplification and some improved sensitivity and ability to isolate specific nucleotide sequences, Mullis also teaches away from Applicants' invention. Specifically, Mullis teaches:

> The present invention obviates the need for extensive purification of the product from a complicated biological mixture.

(Col. 2, lines 32-34). Mullis reaffirmed this teaching later in the disclosure:

> It is not necessary that the sequence to be amplified be present initially in a pure form; it may be a minor fraction of a complex mixture ... or a portion of a nucleic acid sequence due to a particular microorganism which organism might constitute only a very minor fraction of a particular biological sample.

(Col. 5, lines 49-56). Plainly, Mullis teaches that the amplification method of his invention does not include purification before amplification and, in fact, does not require purification. Thus, Mullis teaches away from Applicants' invention.

At the same time, however, Mullis recognized that non-target background nucleic acids might also be amplified in addition to the intended target nucleic acids (See Example 10 at Col. 25, line 23). To the extent Mullis recognized this as a problem, Mullis taught the use of nested sets of primers to decrease this background. Mullis did not disclose or suggest Applicants' method for reducing this background but proposed another method instead. Thus, Mullis' own disclosure belies the Examiner's conclusion of obviousness.

The secondary references do not bridge the gap between Mullis' disclosure and

Applicants' invention. Moss, Stabinsky and Engelhardt do not disclose or suggest amplification of or detection of amplified nucleic acids. Accordingly, none of them even considers problems such as noise resulting from amplification of nonspecifically bound nucleic acids or solutions to such problems. None of them discloses any reason to ignore the teachings of Mullis that Mullis' invention "obviates the need for extensive purification of product from a complicated biological mixture" (U.S. No. 4,683,202 at Col. 2, lines 32-34). Thus, Moss, Stabinsky and Engelhardt do not disclose or suggest Applicants' invention alone or in combination with Mullis.

Similarly, Ranki does not disclose or suggest amplification of nucleic acids. Neither does Ranki consider the significance of nonspecifically bound nucleic acids to an amplification process. Thus, Ranki too does not disclose or suggest Applicants' invention alone or in combination with Mullis.

Josephson and Schroder were cited for disclosing their methods for magnetic separations. However, these references do not address amplification of nucleic acids, problems associated therewith or solutions thereto. They do not disclose or suggest Applicants' invention alone or in combination with Mullis and the other cited references.

Wood, Noyes and Shih were cited as cumulative of Moss, Stabinsky and Engelhardt and not for any new teaching absent from these previously discussed references. Applicants' own review of Wood, Noyes and Shih found no additional teaching which renders Applicants' invention unpatentable alone or in combination with the other cited references.

The Examiner cited numerous references in attempting to establish a prima facie case of obviousness against Applicants' claimed invention. Although Applicants think it plain that the Examiner did not make out a valid prima facie case, Applicants believe the shortcomings of the Examiner's arguments become more apparent when the actions of those skilled in the art are considered. As demonstrated herein, those with skill in the art have incorporated target amplification by PCR into hybridization techniques and have recognized that such processes are more problematic to use than touted by Mullis (U.S. No. 4,683,202) and do require measures to avoid amplification of nucleic acids other than the designated targets. Those with skill in the art did not combine the teachings of the cited references in the manner proposed by the Examiner to obtain Applicants' claimed

invention. Applicants submit the inability of those in the art to resolve problems attendant to the use of PCR in hybridization assays as Applicants have done is compelling evidence of the unobviousness of Applicants' invention. This is discussed more fully below.

As discussed previously, Applicants' invention principally serves to enhance the sensitivity of nucleic acid hybridization assays utilizing target amplification. Targets can be amplified by a number of ways including PCR. Applicant's invention enhances sensitivity by eliminating from the amplification medium extraneous (nonspecific) nucleic acids which might otherwise be amplified by PCR thereby introducing noise into the assay. The problem of nonspecific amplification is real as evidenced by the reports in the references enclosed herewith. For example, as noted at page 1154 of Schochetman.[2] (Appendix 3):

> [PCR] amplified sequences of target DNA can be detected by a variety of methods. If enough amplified DNA is present, it can be visualized after gel electrophoresis and ethidium bromide staining; however, this method cannot provide definitive identification. ... This is particularly true if the target sequence initially is present in extremely small quantities, a situation that may lead to amplification of some nonspecific sequences and yield too little specific DNA to be visualized. (emphasis added)

Similarly, Vosberg noted at page 4 (Appendix 4):

> However, uncontrolled biochemical sample compositions and a high degree of DNA and RNA complexity have the disadvantage of reducing reaction specificity .... Random primer target interactions cannot be excluded.

Vosberg proposed other methods for enhancing such specificity.

At page 144 of PCR Protocols (Appendix 5), the authors urged Judicious Selection of Controls:

> The cloning of amplified product is a case in point. Often, the amount of target generated from an amplification is insufficient for direct cloning and requires reamplification of the target. To minimize reamplification of nonspecific products, the band of interest is first separated on a gel, excised, eluted ... and used to reseed a subsequent amplification. Each of these additional steps can potentially result in cross-contamination and thereby jeopardize the authenticity of the result. (emphasis added)

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[2]The cited passages are marked for identification in the appendices.

Coutlée (Appendix 6) reported similar concerns. In discussing the use of Taq DNA polymerase at page 246, Coutlée reported: "Increased specific and non-specific amplifications were observed with higher quantities of Taq and longer extension times." In discussing identification of amplified products using radio-labelled deoxy-nucleotide triphosphates, Coutlée also noted that "nonspecific bands can sometimes comigrate at the same level as the specific band" (at page 247).

Finally, in U.S. No. 5,374,524, to Miller (Appendix 7) confirms:

> A disadvantage of [PCR] is that the detection of the nucleic acids produced, using a direct assay method, is complicated in that the amplification process can produce nucleic acid sequences which are not faithful copies of the original nucleic acid which was to be copied. These erroneous nucleic acid sequences can provide false positives in the assay which increase the background noise and thus decrease the sensitivity of the entire method.

(See Col. 2, lines 2-10). Miller's solution to this problem is to apply sandwich hybridization techniques following amplification by PCR. Applicants submit that utilization of their invention and target purification prior to amplification overcomes the same concerns identified by Miller and permits detection by other methods in addition to sandwich hybridization. Thus, Applicants' invention provides a more versatile solution to the problems identified by Miller and the other skilled workers cited herein.

Applicants submit that the problem of nonspecific binding is real and substantial for assays seeking ever greater sensitivity. Moreover, as evidenced by those working with PCR, Mullis is simply wrong in stating that PCR eliminates the need for extensive purification.

Applicants also submit the Examiner has overlooked certain advantages in the invention which demonstrate unobviousness. In particular, Applicants' invention simplifies the selection of primers for PCR. As noted by Cahill at page 1483 (Appendix 8), it is well known that "[p]rimer optimization and selection is an empirical process. Each primer pair must be evaluated for sensitivity and specificity by use with actual samples." However, Applicants' invention permits more general primers to be used in the PCR process thereby avoiding the concerns expressed earlier and, at the same time, simplifying many PCR

applications. This is discussed more fully at page 56, lines 22-33 of Applicants' specification.

Applicants' invention overcomes the undeniable problem of nonspecific amplification in PCR based amplification. The invention provides the additional and unexpected advantage of enabling the use of more general primers in the PCR amplification and, therefore, assays utilizing PCR amplification. Applicants submit the Examiner's prima facie case of obviousness reflects more an improper hindsight determination based on Applicants' own teachings than a proper evaluation of what the cited references do teach and those with skill in the art have found to be true. Applicants submit their claimed inventions are patentable and in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

Norval B. Galloway

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Norval B. Galloway
Registration No. 33,595
Amoco Corporation
3100 Woodcreek Drive
Downers Grove, IL 60515
(708) 271-7427

"Express Mail" mailing label number EM256990425 US
Date of Deposit December 5, 1995
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Anna Evans
December 5, 1995